

The Effect of Addition Parsnip Herb and its Extract on Momtaze Hamburger Shelf Life

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Abstract

The antioxidant effects of different forms of parsnip in beef burgers during storage for 9 days at 4°C were studied. Beef burgers were treated with 0.3% parsnip powder, 0.4% parsnip powders, 0.25 and 0.35% ethanol extracts of parsnip and 0.25 and 0.35% aqueous extracts of parsnip as well as the results were compared to beef burgers without any additive (control). Oxidation tests such as the determination of TBA value, peroxide index, pH, anisidine value, and conjugated dienes and TOTOX value were determined at a gap of 3 days interval for a period of 9 days. Experiments were performed in a factorial form in a completely randomized design with the software of SPSS version 16.0. Incorporation of different forms of parsnip decreased ($P < 0.05$) the pH value of the products. In the end of storage, the TBA value of treatments was lower than control. The results show that powder, ethanol extracts and aqueous extracts of parsnip are very effective against lipid oxidation and have potential as natural antioxidants in beef burgers. Among the different forms of parsnip, powder (0.3 and 0.4%) and aqueous extracts of parsnip (0.25 and 0.35%) were more efficient than other treatments.

Keywords: Beef burger; Lipid oxidation; Natural antioxidant; Parsnip; TBA value

Introduction

Nowadays, more and more convenience foods, such as beef burgers, are being consumed in restaurants. These products should contain 20-30% of fat to give the desirable flavour and texture. The high content of unsaturated fatty acids in foods, such as beef burgers, decreases the oxidative stability during storage and cooking processing. Reducing the fat level in burgers is difficult and results in the reduction of some of its sensorial properties such as tenderness and flavour intensity. In meat and meat products there is a high risk of quality loss due to oxidation. Lipid oxidation in meat and meat products leads to rancid taste and off flavour and development of many different substances from which some have even adverse effects to human health e.g. (Ames et al., 1993).

Antioxidants are added to fresh and processed meat to prevent oxidative rancidity, to delay development of off-flavours, and to improve colour stability. They can be grouped into natural antioxidants and synthetic antioxidants, with the latter including, for example, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ). Both types of antioxidants play a very important role in the food market, but the trend is to focus on the healthiness of food products, therefore there is an increasing preference for natural products over synthetic ones. Extensive use of synthetic antioxidants and prolonged ingestion may be associated with the development of cancer (Sasse et al., 2009). In the recent years a lot of research has been carried out evaluating these natural substances as antioxidative additives in food products leading to novel combinations of antioxidants and the development of novel food products. The high

antioxidant capacity of these plant parts is particularly due to their content of different phenols, anthocyanins and ascorbic acid, which can act as radical scavengers (Girones-Vilaplana et al., 2012). In addition to their antioxidative capacity, many of these natural substances have positive effects in the human body and documented health benefits and are therefore highly appreciated food additives. By using natural antioxidants, there is a potential advantage of reducing or replacing synthetic ones, reducing the production of off-flavours, with a greater acceptance by the consumer (Karaaslan et al., 2011). Consequently, the search for natural additives, especially of plant origin, has been notably increased in recent years. Compounds obtained from natural sources such as grains, oilseeds, spices, fruit and vegetables have been investigated (Meyer et al., 2002).

Parsnip (*Pastinaca sativa* L.) is a root that belongs to the Apiaceae family. It is native from Europe and Asia and used for culinary purposes e.g. as an ingredient in infant food and to feed livestock (Hutschenreuther, 2009). Parsnip roots have a high content of dietary fibre. Some studies reported values of 4.7–4.9% (wet basis) dietary fibre in parsnip (Southgate, 2001). Parsnip is valuable as herbal raw material. Its herb, roots, and fruits contain many active substances such as essential oils, flavonoids, acetylene, and furanocoumarin compounds (Dyduch and Wolski, 1996). Parsnips are known to contain at least six furanocoumarins namely, xanthotoxin, bergapten, isopimpinellin, angelicin, psoralen and imperatorin (Desjardins et al., 1989; Ekiert and Gomólka, 2000). Due to parsnip herb has active substances and natural antioxidants, such as; flavonoids and vitamin C, in this study we use from parsnip herb, its ethanol extract and its aqueous extract as natural antioxidant sources in beef burgers to evaluate the effect of those on oxidative stability, physicochemical and sensory properties of beef burger treatments.

To our knowledge, there are no previous reports on the antioxidant activity of parsnip herbs, ethanol extracts and aqueous extract in food industry, especially meat and meat products industry, or their effects on sensory properties. The aim of this study was to assess the effectiveness of parsnip on lipid stability during storage for increasing the shelf life of the beef burgers and studying the sensory properties of them.

Material and methods

Material

2-thiobarbituric acid (TBA), 1-butanol, acetic acid glacial, chloroform, potassium iodide, sodium tiosulphate, isooctane, anisidine were purchased from Sigma-Aldrich.

Dressed and deboned beef meat procured from local market, Damghan, Iran and packed in clean Polyethylene bags. Meats are quickly conveyed to the laboratory of Food Science and Technology and stored at 4 °C until further use. Other additives which used in this project were onion, salt (sodium chloride), bread crumbs, wheat flour, red pepper, garlic powder, turmeric and nutmeg that prepared from local market. Fresh parsnips were collected from Chalos regions in spring season. They dried in oven at 50 °C for 4 days. After drying, fine powder of parsnip was prepared using mixer grinder (Moulinex, AW5 Model, France) and stored at 4°C until use.

Preparation of parsnip extracts

Fifty grams of parsnip powder were soaked in 1 L of 70% (v/v) ethanol/distillate water and mixed for 24 h at room temperature using an overhead stirrer (Heidolph MR Hei-standard, Germany). After vacuum filtering (Platinum, JB industriels INC, USA), the obtained extracts were concentrated under reduced pressure in a water bath set at 45°C using a rotary evaporator (Heidolph, Laborata 4000-efficient, Germany). The extracts were then stored at 4°C till further use.

Hamburger manufacture

Hamburgers (~85 gr) were manufactured with fresh beef meat. Meat was ground using meat grinder (Panasonic, MK-G40, Iran) by a 3 mm plate. Seven batches were manufactured according to

related formulation: a control batch without parsnip, with 0.3% powder parsnip, with 0.4% powder parsnip, with 0.25% ethanol extract of parsnip, with 0.35% ethanol extract of parsnip, with 0.25% aqueous extract of parsnip and 0.35% aqueous extract of parsnip (Table 1). After mixing the material (mentioned in Table 2.1) the burgers were shaped into plates with a diameter of 10 cm and a height of ~1 cm. The hamburgers were placed in laminated bags of low permeability and it was to be used and analyzed after 1,3,6 and 9 days of storage at 4 °C. From each batch three replicates were made.

Table 1: Formulation for beef burgers made in present study

| Ingredients (%) | Burger 1 | Burger 2 | Burger 3 | Burger 4 | Burger 5 | Burger 6 | Burger 7 |
|----------------------------|----------|----------|----------|----------|----------|----------|----------|
| Meat | 61.5 | 61.5 | 61.5 | 61.5 | 61.5 | 61.5 | 61.5 |
| Onion | 24 | 23.7 | 23.6 | 23.75 | 23.65 | 23.75 | 23.65 |
| Salt | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 |
| Red pepper | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Bread crumbs | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Wheat flour | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Garlic powder | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 |
| Turmeric | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 |
| Nutmeg | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 |
| Parsnip powder | - | 0.3 | 0.4 | - | - | - | - |
| Ethanol extract of parsnip | - | - | - | 0.25 | 0.35 | - | - |
| Aqueous extract of parsnip | - | - | - | - | - | 0.25 | 0.35 |

Burger1: control burger; Burger2: with 0.3% parsnip powder; Burger3: with 0.4% parsnip powder; Burger4: with 0.25% ethanol extract of parsnip; Burger5: with 0.35% ethanol extract of parsnip; Burger6: with 0.25% aqueous extract of parsnip; Burger7: wit 0.35% aqueous extract of parsnip.

Analytical procedure

pH measurement

The pH value was recorded using a pH meter (3510 pH Meter, Jenway, England) and based on the method of Dzudie et al. (2004).

Determination of peroxide value (POV)

Peroxide value (POV) was determined according to the AOAC International (1998) with the iodometric method. POV was calculated and expressed as milli equivalent peroxide per kg of sample.

TBA value

The 2-thiobarbituric acid (TBA) assay was carried out according to the procedure of Natseba et al (2005). The absorbance measured at 532 nm in 1cm cells against distilled water blank (Jenway 6305, England). TBA value was expressed as mg malonaldehyde per kg of beef burger.

Conjugated dienes (CDs)

Lipid was extracted from the beef burger samples using a modified method of Egan et al. (1981). To determination of CDs, approximately 70 mg of lipid were weighed into a 100 ml volumetric flask and filled up to volume with isooctane. The flasks were thoroughly shaken to obtain homogeneous distribution. The absorbance was measured using a spectrophotometer (Jenway 6305, England) at 232 nm against a blank of isooctane. CDs were calculated in accordance with the Ti 1a-64 methods (AOCS, 2003), using the formula: $CD = 0.84 [(As/bc) - K0]$. As is the absorbance

at 232 nm, *b* is the cuvette length (cm), *c* is the sample concentration in isooctane (gr/L), and *K₀* represents the absorptivity by acid or ester groups (0.07 for esters, 0.03 for acids).

Anisidine value

The anisidine value is defined as 100 times the optical density measured in a 1 cm cell of a solution containing 1 g of the substance to be examined in 100 ml of a mixture of solvents and reagents according to the method described by Dieffenbacher and Pocklington (1987).

Totox Value

The Totox value was obtained from the potentiometric readings of PV and AV according to AOCS method (AOCS, 1998) using the following equation:

$$\text{Totox value} = 2\text{PV} + \text{AnV}$$

Sensory evaluation

Representative samples of the different burger formulations were grilled at 170° C for 10 min. The flavour, odour, texture and overall acceptability scores of the burgers were determined by 30 panellists at 3 day of storage. A seven-point hedonic scoring scale (7=excellent; 6=very good; 5=good; 4=moderate; 3=slightly bad; 2=bad; 1=very much bad) was employed for evaluation of burgers.

Statistical analysis

All measurements were carried out in triplicate (*n* = 3), and results were subjected to one way analysis of variance (ANOVA) in a factorial form in a completely randomized design using SPSS software (SPSS 16.0). Differences between means were determined with Duncan's Multiple Range Test, and significance was defined at *p* < 0.05.

Results and discussion

pH

Table 2: Evaluation of pH in beef burger treatments stored under refrigeration (4±1 °C) during 9 days.

| Treatments/Times | 1 day | 3 days | 6 days | 9 days |
|------------------------------|-----------------|-----------------|-----------------|-----------------|
| Control | 4.41 ± 0.01B,c | 4.39 ± 0.01C,c | 4.39 ± 0.18C,a | 4.49 ± 0.17A,a |
| Burger+0.3% powder | 4.57 ± 0.00A,ab | 4.41 ± 0.03B,ab | 4.28 ± 0.02D,ab | 4.35 ± 0.01C,b |
| Burger+0.4% powder | 4.68 ± 0.00A,b | 4.43 ± 0.06B,ab | 4.28 ± 0.05D,ab | 4.38 ± 0.01C,b |
| Burger+ 0.25 ethanol extract | 5.06 ± 0.01A,a | 4.60 ± 0.02B,b | 4.10 ± 0.03D, c | 4.30 ± 0.02C,c |
| Burger+0.35 ethanol extract | 5.06 ± 0.00A,a | 4.82 ± 0.02B,a | 4.21 ± 0.05D,b | 4.36 ± 0.04C,b |
| Burger+0.25 aqueous extract | 4.50 ± 0.00A,ab | 4.38 ± 0.01B, c | 4.30 ± 0.02C,ab | 4.50 ± 0.00A,a |
| Burger+0.35 aqueous extract | 4.63 ± 0.06A,b | 4.41 ± 0.02B,ab | 4.22 ± 0.01C,b | 4.34 ± 0.15B,bc |

*Results are expressed as means ± standard deviations of the three replicates. ^{A-D}Averages with different letters in the same column indicate significant differences (*p* < 0.05). ^{a-c} Averages with different letters in the same row indicate significant differences (*p* < 0.05).

According to Statistical results, Storage time, concentration and type's additive have significant effect on meat value of pH. The initial pH value ranged from 4.41 (in control samples) to 5.06 (in ethanol extract of parsnip-formulated samples). Although at the first of storage, the pH value was found to be lower (4.41 ± 0.01) in control samples than the other samples and other treatments were having the pH in the range between 4.50 ± 0.00 and 5.06 ± 0.01, at the end of storage time, the pH value of control sample was higher than the others (Figure 1).

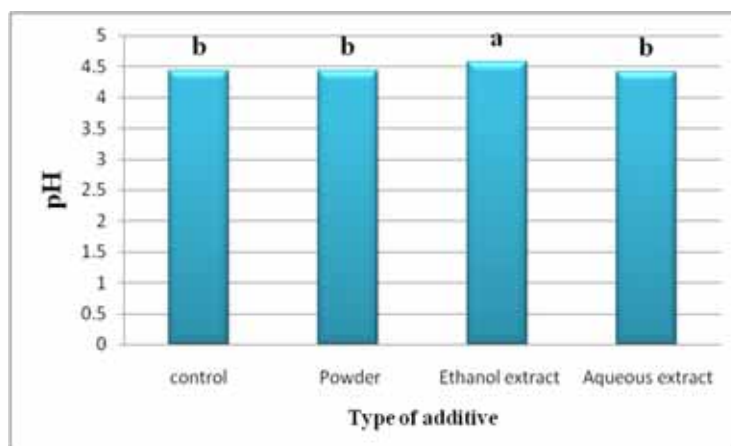


Figure 1: Effect of types on pH of burgers at refrigeration (4°C).

***a-b: Significant differences between pH values ($p < 0.05$)**

In all types of burgers, storage time had a significant ($P < 0.05$) effect on the pH values (Figure 2). The mean value of pH decreased from 1 to 6 days storage. After that it increased until 9 days storage. These changes may be due to the usage of amino acids by bacteria, released during the protein break down as the stored glucose has depleted. Accumulation of ammonia and the product of amino acid degradation results in the pH increase. When bacteria grows in meat and meat products, at first, it uses from glucose (from fermentation way) as a source of nutrition and produces acid products, that can be decreased the pH value. When sugar was finished, bacteria use from amino acids and release ammonia and pH would rise (Gill, 1983). Babji et al., (2000) associated high pH values ($pH > 6.0$) in minced goat meat with high Enterobacteriaceae counts.

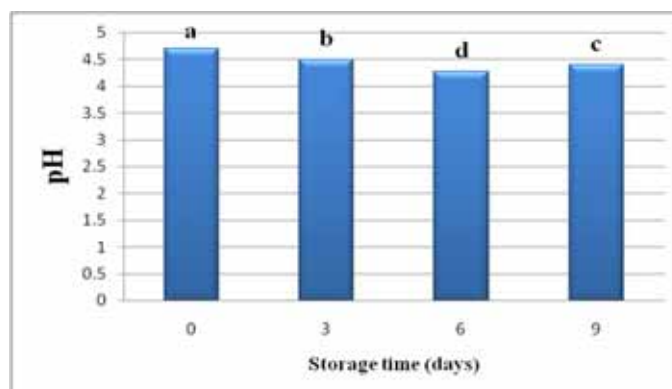


Figure 2: Effect of storage time on pH of burgers at refrigeration (4°C)

***a-d: Significant differences between pH values ($p < 0.05$).**

This result showed that antibacterial activity of extract may be affected by different concentrations. Although storage time had a significant effect on pH, differences of 0.4 (in treatments) were not marginal and not significant on a practical level.

Peroxide value (POV)

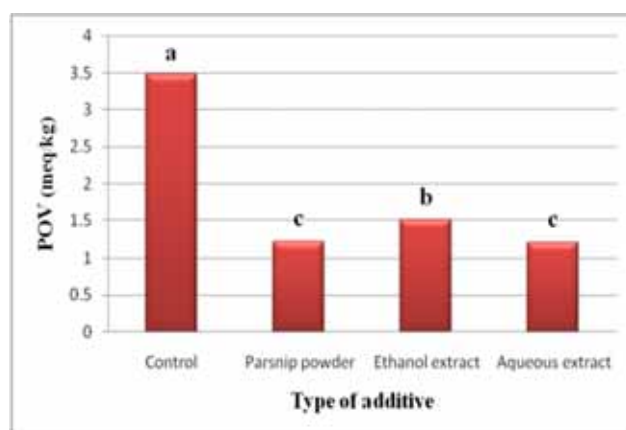
Peroxides are the primary products of oxidation. However, since they are relatively short lived, their usefulness as oxidation indicators, is limited to an early stage of rancidity development. As oxidation proceeds, peroxides break down to aldehydes or combine with proteins (Woyewoda et al., 1986). Table 3 show the peroxide values of beef burgers during refrigerated storage at 4°C.

Table 3: Changes of peroxide (meq/kg) in burgers during 9 days of storage at refrigeration

| Treatments/Times | 1 day | 3 days | 6 days | 9 days |
|------------------------------|-------|--------------|---------------|---------------|
| Control | 0D,a | 1.6 ± 0.0A,a | 8.5 ± 0.40B,a | 3.8 ± 0.40C,a |
| Burger+0.3% powder | 0D,a | 1.0 ± 0.0B,b | 3.5 ± 0.40A,c | 2.8 ± 0.40B,b |
| Burger+0.4% powder | 0C,a | 0C,d | 2.1 ± 0.17A,d | 0.3 ± 0.00B,e |
| Burger+ 0.25 ethanol extract | 0D,a | 0.6 ± 0.0C,c | 6.6 ± 0.00A,b | 1.4 ± 0.35B,c |
| Burger+0.35 ethanol extract | 0C,a | 0C,d | 3.4 ± 0.17A,c | 0.1 ± 0.17B,d |
| Burger+0.25 aqueous extract | 0C,a | 0C,d | 3.2 ± 0.17A,c | 1.3 ± 0.30B,c |
| Burger+0.35 aqueous extract | 0D,a | 1.0 ± 0.0C,b | 1.6 ± 0.00A,e | 1.3 ± 0.00B,c |

*Results are expressed as means ± standard deviations of the three replicates. ^{A-D}Averages with different letters in the same row indicate significant differences ($p < 0.05$). ^{a-e}Averages with different letters in the same column indicate significant differences ($p < 0.05$).

Data revealed that the initial POV in all of the treatments were zero. The results also revealed that POV had gradually increased during storage at 4°C for 6 days, where the highest level was at day 6 varying from 1.6-8.5 (meq/kg fat) for all analyzed beef burgers. In such that 1.6 was for 0.35% aqueous extracts and 8.5 were for control samples. These results are in agreement with those of Alina et al., (2012) who showed that peroxide value increased from 1.885 to 8.470 in chicken sausage samples containing 1.5% of rice bran during nine days of chilled storage. The peroxide value in all treatments tended to increase significantly by the progress of the storage period up to 6 days then decreased suddenly at the end of the storage period. POV tends to increase during the early stages of oxidation, when the rate of hydroperoxides formation is higher than the rate of their decomposition. A significant decrease of POV after an initial increase confirms that peroxides formed in the early stages of oxidation are unstable and highly susceptible to further changes that result in the formation of secondary products of oxidation (Farhoosh and Moosavi, 2009). At the end of storage, Samples treated with 0.35% ethanol extract of parsnip (0.1 ± 0.17) and 0.4% parsnip powder (0.3 ± 0.00) showed the lowest POV and the control samples showed the highest POV (3.77 meq/kg fat) (Table 3).

**Figure 3: Effect of type of additives on POV (meq/kg) at refrigerated storage (4°C).**

*0= control (without parsnip), 1= parsnip powder, 2= ethanol extract of parsnip and 3= aqueous extract of parsnip. a-c: Significant differences between POV ($p < 0.05$).

Therefore, it could be stated that the treatments containing 0.35% ethanol extract of parsnip and 0.4% parsnip powder have a greater antioxidant activity against peroxide formation than other treatments.

Thiobarbituric acid (TBA)

The thiobarbituric acid (TBA) test was proposed over 40 years ago and is now one of the most extensively used methods to detect oxidative deterioration of fat-containing foods. During lipid oxidation, malonaldehyde (MA), a minor component of fatty acids with 3 or more double bonds, is formed as a result of the degradation of polyunsaturated fatty acids. It is usually used as an indicator of the lipid oxidation process, both for the early appearance as oxidation occurs and for the sensitivity of the analytical method (Kishida et al., 1993).

The effect of the natural extracts and powder of parsnip on lipid oxidation of beef burgers during storage is shown in table 4. The analysis of variance for the TBARS data indicates that the TBA values were significantly affected ($p < 0.05$) by both the storage period (Figure 3.4) and the concentration of extract treatments, and no significant effect was observed for the type of additives and its interaction (kind*concentration) ($p > 0.05$). Initial (day 1) TBA values for 0.35% ethanol extract was significantly lower than others (0.14 ± 0.03 mg MDA/kg). This result suggests that this antioxidant retarded lipid oxidation during and immediately after making.

Table 4: Averages values of TBARS (mg MDA/kg) in burgers under refrigeration storage

| Treatments/Times | 1 day | 3 days | 6 days | 9 days |
|------------------------------|--------------------|--------------------|---------------------|--------------------|
| Control | $0.16 \pm 0.04C,e$ | $0.32 \pm 0.20B,d$ | $0.19 \pm 0.03C,c$ | $1.36 \pm 0.29A,a$ |
| Burger+0.3% powder | $0.83 \pm 0.40A,b$ | $0.71 \pm 0.10B,b$ | $0.27 \pm 0.0D,b$ | $0.55 \pm 0.10C,c$ |
| Burger+0.4% powder | $0.60 \pm 0.10A,c$ | $0.64 \pm 0.11A,b$ | $0.43 \pm 0.017C,a$ | $0.51 \pm 0.09B,c$ |
| Burger+ 0.25 ethanol extract | $1.22 \pm 0.10A,a$ | $0.41 \pm 0.13B,c$ | $0.37 \pm 0.0C,a$ | $0.47 \pm 0.22B,d$ |
| Burger+0.35 ethanol extract | $0.14 \pm 0.03D,e$ | $0.69 \pm 0.06A,b$ | $0.28 \pm 0.03C,b$ | $0.55 \pm 0.05B,c$ |
| Burger+0.25 aqueous extract | $0.33 \pm 0.06C,d$ | $0.84 \pm 0.19B,a$ | $0.39 \pm 0.03C,a$ | $0.93 \pm 0.15A,b$ |
| Burger+0.35 aqueous extract | $0.34 \pm 0.06B,d$ | $0.55 \pm 0.04A,c$ | $0.29 \pm 0.09B,b$ | $0.48 \pm 0.09A,d$ |

*For each treatment, averages followed by different lowercase letters in the same column differ significantly ($p \leq 0.05$) by the Duncan test. For each storage time, averages followed by different capital letters in the same row differ significantly ($p \leq 0.05$) by the Duncan HSD test.

At the end of storage, the control treatment (1.36 ± 0.29 mg MDA/kg meat) had significantly higher TBARS values ($p < 0.05$) when compared to other treatments with antioxidants (Table 4). According to Al-Kahtani et al. (1996), meat products can be considered well preserved in regards to oxidative changes, when they had less than 3 mg MDA/kg sample. Thus, all of treatments showed values lower than 3 mg MDA/kg meat, indicating that the samples were suitable for consumption. For the storage period, the TBARS values increased over time with an average of 0.51 mg MDA/kg meat at the beginning of the experiment and an average of 0.70 mg MDA/kg meat at the end of the experiment (nine days). Similar results were reported by Brannan (2008) that observed an increase in the TBARS values of chicken meat during refrigerated storage. Similarly, the study of Brannan (2008), with chicken meat, found significant reductions in TBARS values in treatments with the addition of grape extract when compared to control.

In the present study, TBA of burgers in the control started to increase significantly ($p < 0.01$) from 0.16 (day 1) to reach 1.36 mg MDA/kg meat at the end of storage period. While, the maximum values for beef burgers treated with aqueous and ethanol extracts, and powders of parsnip were 0.93 and 0.55 mg MDA/kg meat at the end of refrigerated period, respectively. Also, Formanek et al., (2009) and Ibrahim et al., (2011) reported that ginger extract as antioxidant was effective against TBA formation when incorporated into meat during frozen storage.

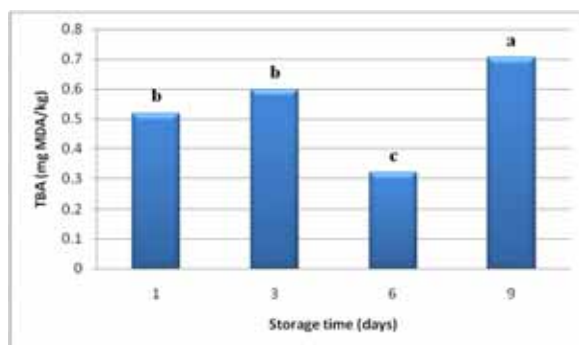


Figure 4: Effect of storage time on TBA value (mg MDA/kg) in beef burger samples
***a-c: Significant differences between TBA values ($p < 0.05$).**

The results demonstrating the efficiency of powder, ethanol extracts and aqueous extracts of parsnip as antioxidants in beef burgers. These results agree with those observed by Rababah et al. (2006) and Shirahigue et al. (2010), who found a reduction in TBARS in chicken meat with grape seed extract during refrigerated storage. Moreover, since TBA values is considered as indicators of rancidity in fat products, Verme and Sahoo (2000) indicate that MDA concentrations between 1.0 and 2.0 mg/kg as threshold values for rancidity.

Conjugated diens

The polyunsaturated fatty acids oxidation occurs with the formation of hydroperoxides. Immediately after peroxides have been formed, the non-conjugated double bonds present in natural unsaturated lipids suffer a rearrangement generating conjugated dienes (CD), which absorb at 232 nm (Gertz et al., 2000). In accordance with this, the instability of peroxide molecules may also explain the decrease in PV during advanced stages of rancidity, so that breakdown into smaller molecules compounds associated with oxidation of lipids would be expected to occur (Suleiman et al., 2006). The results from the analysis of conjugated dienes in all burger treatments are presented in Table 5. According to the results of variance analysis for conjugated dienes, values were significantly affected ($p < 0.05$) by type, concentration and time of storage (Figure 5). The evaluation of the conjugated diene results showed that at the first day, the sample with 0.35% aqueous extract of parsnip has the lowest value, and the highest value of conjugated dienes was for 0.3% parsnip powder sample.

Table 5: The mean value of conjugated dienes (CDs) (%) for burgers during refrigeration storage.

| Treatments/Times | 1 day | 3 days | 6 days | 9 days |
|------------------------------|--------------------|--------------------|--------------------|--------------------|
| Control | $0.68 \pm 0.05A,c$ | $0.74 \pm 0.05A,a$ | $0.20 \pm 0.01B,a$ | $0.15 \pm 0.01C,c$ |
| Burger+0.3% powder | $1.22 \pm 0.03A,a$ | $0.10 \pm 0.02C,d$ | $0.13 \pm 0.01C,d$ | $0.34 \pm 0.06B,b$ |
| Burger+0.4% powder | $0.56 \pm 0.04A,d$ | $0.12 \pm 0.01C,d$ | $0.14 \pm 0.02B,c$ | $0.10 \pm 0.01C,d$ |
| Burger+ 0.25 ethanol extract | $1.02 \pm 0.08A,a$ | $0.14 \pm 0.06D,d$ | $0.20 \pm 0.02C,a$ | $0.28 \pm 0.01B,c$ |
| Burger+0.35 ethanol extract | $0.87 \pm 0.05A,b$ | $0.22 \pm 0.01C,b$ | $0.14 \pm 0.00D,c$ | $0.63 \pm 0.01B,a$ |
| Burger+0.25 aqueous extract | $0.84 \pm 0.02A,b$ | $0.18 \pm 0.01B,c$ | $0.16 \pm 0.01B,b$ | $0.09 \pm 0.01C,d$ |
| Burger+0.35 aqueous extract | $0.45 \pm 0.03A,d$ | $0.18 \pm 0.01B,c$ | $0.14 \pm 0.06B,c$ | $0.03 \pm 0.06C,d$ |

*Results are expressed as means \pm standard deviations of the three replicates. A-D Averages with different letters in the same row indicate significant differences ($p < 0.05$). a-d Averages with different letters in the same column indicate significant differences ($p < 0.05$).

After 9 days of refrigeration, burgers treated with aqueous extracts were the most resist to oxidation, as evidenced by the lowest value of 0.03-0.09%, followed by 0.4% parsnip powder, control, 0.25% ethanol extract, 0.3% powder of parsnip and 0.35% ethanol extract. The increase in conjugated diene formation in control was observed until the 3 days of the period tested (9 days of refrigeration) and then decreased until the end of storage. Aqueous extracts and 0.4% powder of parsnip, presented decrease significantly ($p < 0.05$) until the end of storage (9 days). According to the mechanism that suggested for lipid oxidation the conjugated dienes formation is faster than the TBARs formation during refrigeration of beef burgers.

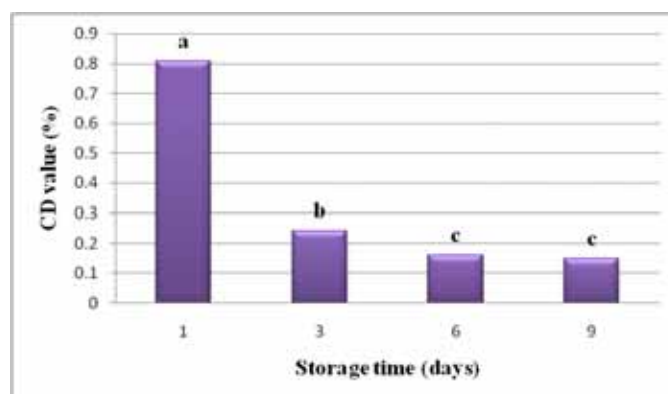


Figure 5: Effect of storage time on CD values for burger treatments.

***a-c: Significant differences between CD values ($p < 0.05$).**

However, the results from the present study suggested that addition of aqueous extracts and 0.4% powder of parsnip to burgers were able to increase the resistance to oxidation, in comparison with the control group and other treatments, as shown by the lower conjugated diene contents presented in antioxidant groups after storage time (9 days). This is in agreement with previous studies, in which it was reported that the concentration of conjugated dienes significantly increased in cooked pork patties treated with antioxidants, over the refrigeration period (Juntachote et al., 2006; Lee et al., 2010).

Anisidine value (AnV)

P-anisidine value was analyzed to determined secondary product of lipid oxidation. P-anisidine value is mainly a measure of 2-alkenals and 2,4-dienals in animal fat and vegetable oil. As the peroxides in an oxidising fat or oil are transitory, p-AV was measured in addition to PV in order to gain a measure of the peroxide breakdown products present in the oil. Indeed, the rate of increase in the PV of the extracted oil samples decreased as the storage experiment progressed, which reflects decomposition of peroxides to secondary oxidation products (zhang et al., 2010). At table 6, the changes recorded in AnV for burger samples under refrigerated storage were seen.

Table 6: The mean values of anisidine for burgers during refrigeration storage

| Treatments/Times | 1 day | 3 days | 6 days | 9 days |
|------------------------------|--------------------|---------------------|---------------------|---------------------|
| Control | $0.50 \pm 0.03D,c$ | $16.60 \pm 1.02A,d$ | $10.49 \pm 1.53C,d$ | $14.04 \pm 3.10B,b$ |
| Burger+0.3% powder | $1.17 \pm 0.21D,b$ | $17.56 \pm 0.16A,d$ | $9.90 \pm 1.83C,d$ | $11.90 \pm 1.07B,c$ |
| Burger+0.4% powder | $3.46 \pm 0.23D,a$ | $34.55 \pm 0.10A,a$ | $29.31 \pm 0.09B,a$ | $16.42 \pm 0.78C,a$ |
| Burger+ 0.25 ethanol extract | $0.25 \pm 0.30D,c$ | $27.59 \pm 0.33A,b$ | $11.04 \pm 1.58C,d$ | $14.34 \pm 0.98B,b$ |
| Burger+0.35 ethanol extract | $0.37 \pm 0.03D,c$ | $23.00 \pm 0.03A,c$ | $19.11 \pm 3.76B,b$ | $13.94 \pm 0.64C,b$ |
| Burger+0.25 aqueous extract | $0.25 \pm 0.01C,c$ | $16.63 \pm 1.57A,d$ | $16.53 \pm 0.90A,c$ | $11.54 \pm 1.03B,c$ |
| Burger+0.35 aqueous extract | $3.00 \pm 0.25D,a$ | $15.11 \pm 0.18B,d$ | $18.33 \pm 0.91A,b$ | $12.74 \pm 1.09C,c$ |

* Results are expressed as means \pm standard deviations of the three replicates. ^{A-D}Averages with different letters in the same row indicate significant differences ($p < 0.05$). ^{a-d}Averages with different letters in the same column indicate significant differences ($p < 0.05$).

It can be observed that at the first day after making burgers, anisidine values in burgers containing 0.4% parsnip powder (3.46 ± 0.23) and 0.35% ethanol extract (3.0 ± 0.25) were the highest amounts and in burgers with 0.25% aqueous extract (0.25 ± 0.01) and 0.25% ethanol extract (0.25 ± 0.3) were the lowest amounts.

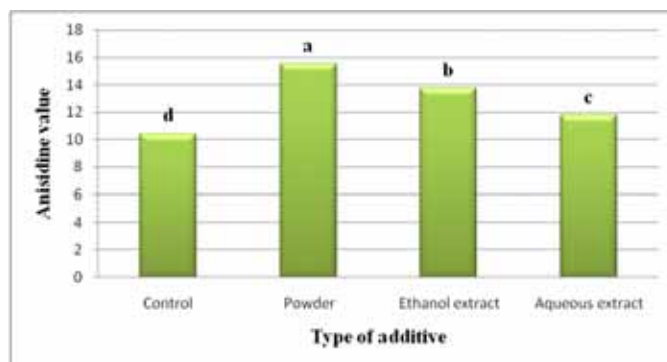


Figure 6: Effect of additive type on anisidine value in beef burger samples

***a-d: Significant differences between anisidine values ($p < 0.05$).**

According to results of variance analyses for anisidine value, values were significantly affected ($p < 0.05$) by kind (Figure 4.6), concentration and time of storage. At the end of storage (9 days), p-AV of samples with 0.4% parsnip powder (16.42 ± 0.78), control burgers (14.04 ± 3.1) and burgers with 0.25% ethanol extract were significantly ($p < 0.05$) higher than other samples. The result of this study showed that the p-anisidine values in burgers with 0.25% aqueous extract (11.54 ± 1.03) and 0.3% parsnip powder were significantly ($p < 0.05$) lower than in control burgers (14.04 ± 3.1) (Table 6).

Total oxidation value (TOTOX value)

The use of PV and p-AV together provides a comprehensive overview of the oxidation process in oils. This is a mathematical prediction of oxidative stability and the value is calculated as TOTOX value. TOTOX value was used as an indication of overall oxidative stability and was correlated with the extent of oil deterioration (De Abreu et al., 2010). TOTOX value for treatments is given in table 7, and shows the TOTOX value in control, burgers with 0.35% ethanol extract, 0.25% and 0.35% aqueous extract increasing as the storage time is increased till 6 days.

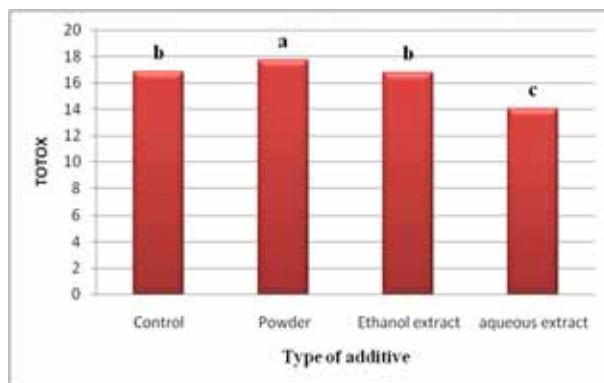


Figure 7: Effect of additive type on TOTOX value in beef burger samples

The calculated value of the TOTOX index after preparation was analogous to the determined AnV value, since POV was assayed to be zero. At the end, the lowest TOTOX values by supplementation with aqueous extract of parsnip to a level of 0.25% were recorded.

Table 7: The mean value of TOTOX index for burgers during refrigeration storage

| Treatments/Times | 1 day | 3 days | 6 days | 9 days |
|------------------------------|----------------|-----------------|-----------------|-----------------|
| Control | 0.48 ± 0.05C,c | 19.63 ± 1.32B,c | 27.55 ± 2.09A,b | 19.71 ± 0.7B,a |
| Burger+0.3% powder | 1.16 ± 0.21C,b | 19.56 ± 0.16A,c | 15.57 ± 1.21B,c | 16.91 ± 2.06B,b |
| Burger+0.4% powder | 3.46 ± 0.23C,a | 34.55 ± 0.10A,a | 33.45 ± 0.21A,a | 17.02 ± 0.78B,b |
| Burger+ 0.25 ethanol extract | 0.25 ± 0.03D,d | 28.79 ± 0.33A,b | 24.24 ± 1.58B,b | 17.14 ± 0.89C,c |
| Burger+0.35 ethanol extract | 0.37 ± 0.03D,d | 23.00 ± 0.03B,b | 25.91 ± 3.58A,b | 14.14 ± 0.87C,c |
| Burger+0.25 aqueous extract | 0.25 ± 0.01D,d | 16.63 ± 1.57B,d | 22.93 ± 0.71A,b | 14.14 ± 0.67C,c |
| Burger+0.35 aqueous extract | 3.01 ± 0.25C,a | 17.11 ± 0.18B,d | 21.53 ± 0.91A,b | 17.83 ± 1.40B,b |

*Results are expressed as means ± standard deviations of the three replicates. A-DAverages with different letters in the same row indicate significant differences ($p < 0.05$). a-d Averages with different letters in the same column indicate significant differences ($p < 0.05$).

Inhibition of oxidation processes by 0.25% aqueous extract of parsnip was observed during all of 9 days storage times (Table 7). According to variance analysis, type (Figure 7), concentration and storage time were affected on TOTOX value in beef burger treatments. The lower amount of TOTOX value was seen in aqueous extract.

Sensory evaluation

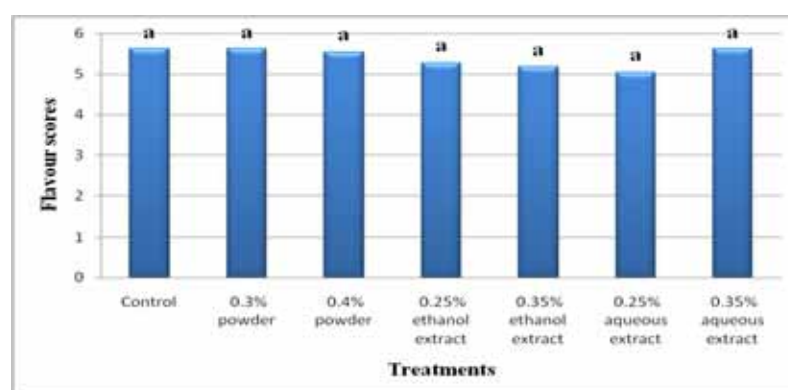


Figure 8: The mean value of flavor scores for burger treatments at 3 days of refrigeration storage.

Flavour is an important meat quality attribute and is strongly associated with the perception of meat products palatability, which is a determinant of whether a consumer will repurchase a specific type of meat product or not (Goodson et al., 2002). According to variance analysis, no difference ($p > 0.05$) was detected in the flavour between the different formulations of burgers (Figure 4.8). Flavour and texture scores of control and the all treatments were almost similar and incorporation of parsnip powder, ethanol extracts of parsnip and aqueous extracts of parsnip did not make marked changes in any of the attributes. Similarly, Banerjee et al., (2012) state that there were no significant differences in the organoleptic characteristics of all the products. Appearance, flavour, texture, juiciness and overall acceptability scores of control nuggets, broccoli powder extract nuggets and BHT nuggets were almost similar and incorporation of broccoli powder extract did not make marked changes in any of the attributes.

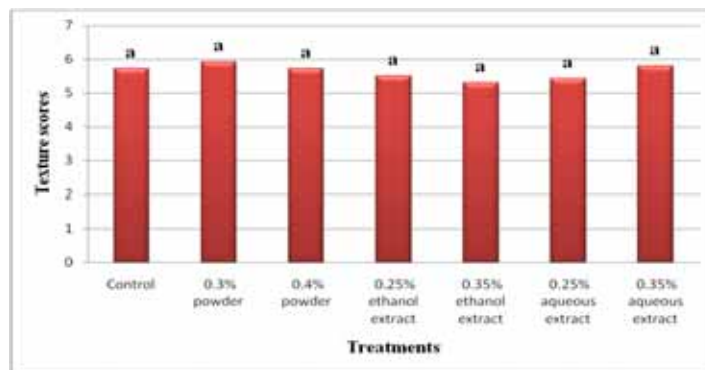


Figure 9: The mean value of texture scores for burger treatments at 3 days of refrigeration storage.

Beef burgers samples had pleasant texture and flavour on the panel test day (day 1). The results showed that the samples treated with and without powder and extracts of parsnip were acceptable from panellists. However, the scores of samples treated with 0.3% parsnip powder were slightly higher than the other samples. The results of variance analysis show that type of additive and concentration had no significant effect on texture and flavour characteristics ($p > 0.05$) (see figures 8 & 9).

The lower acceptability score of 4.63 for odour was for burgers containing 0.35% ethanol extract and 0.25% aqueous extract of parsnip and the higher score was for samples with 0.4% parsnip powder (Figure 10). According to variance analysis, type of additive and concentration had no significant effect on odour characteristic ($p > 0.05$), while interaction of them had significant effect on it ($p < 0.05$).

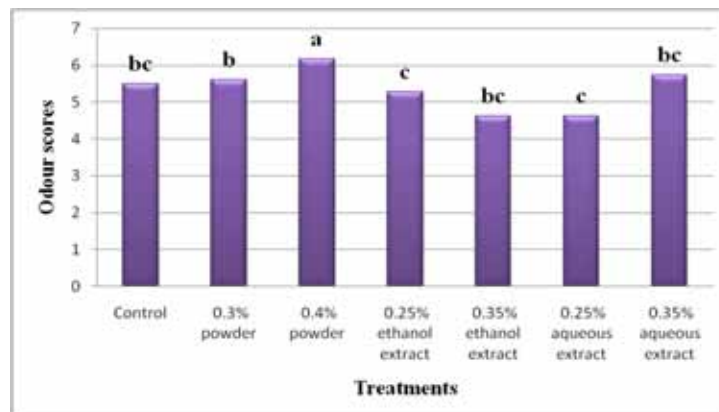


Figure 10: The mean value of odour scores for burger treatments at 3 days of refrigeration storage.

***a-c: indicate significant differences ($p < 0.05$).**

The results of variance analysis show that only type of additive significantly has effect on overall acceptance scores (Figure 11). The burgers with 0.3% parsnip powder (5.87 ± 1.196) and 0.25% aqueous extract of parsnip presented high sensory acceptability, while the lowest score was for burgers with 0.35% ethanol extract of parsnip. However, the panellists accepted all the samples of beef burger (Figure 11).

In general, the sensory evaluation indicated that the use of powder, ethanol extract and aqueous extract of parsnip resulted in a pleasant sensory characteristics beef burgers.

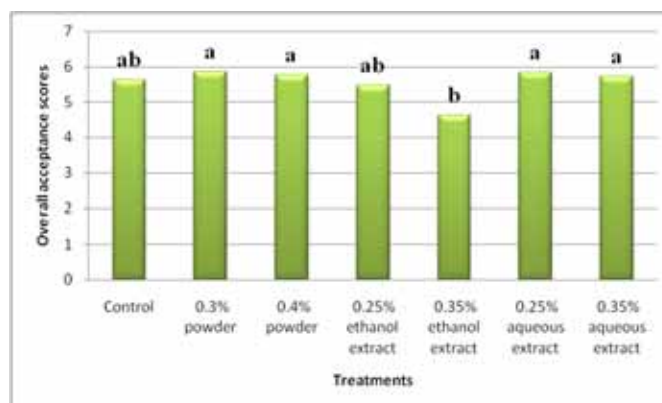


Figure 11: The mean value of overall acceptance scores for burger treatments at 3 days of refrigeration storage.

***a,b: indicate significant differences ($p < 0.05$)**

Conclusion

This study concluded that parsnip powder, ethanol and aqueous extracts of parsnip provide antioxidant benefits to beef burgers during cold storage (4°C) and the effects are concentration dependent. Among the parsnip forms studied, powder and aqueous extracts of parsnip demonstrated the most potent effect, these forms had acceptable scores in sensory evaluation, too. The results showed that the addition of different forms of parsnip could effectively reduce lipid oxidation, maintain or improve sensory attributes and extend the shelf-life of beef burgers during refrigerated storage. Therefore, it is suggested that parsnip, as a natural herb, could be used to extend the shelf-life of meat products, providing the consumer with food containing natural additives, which might be seen more healthful than those of synthetic origin.

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